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The Ongoing Search for Neural Correlates of Consciousness:
Does the P300 Reflect Conscious Perception Or Its
Consequences?

Seminar paper

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Abstract

The Ongoing Search for Neural Correlates of Consciousness: Does the P300 Reflect Conscious Perception Or Its Consequences?

A common scientific strategy towards understanding consciousness is to study neural correlates of consciousness (NCC) for a particular conscious percept. It can be done by contrasting conditions in which subjects are aware and unaware of a particular visual stimulus. However, recent findings have been contradictory and this approach appears not to reveal only the NCC, but also the prerequisites or consequences of consciousness. The goal of the present study was to investigate whether the P300 component often claimed to be a key signature of conscious access might actually rather reflect the consequences of conscious perception. Twenty-one healthy subjects participated in the EEG experiment where most of the stimuli were clearly perceived and only a quartile of trials was associated with not perceiving the target. The visual masking paradigm used no discrimination task, always the same stimulus was presented and the additional task was given together with the target stimulus. Results indicate that trials where subjects reported to have seen the stimulus are associated with a higher P300. Hence, the present data support the theories, which claim that P300 is a marker of conscious perception.

Keywords: neural correlates of consciousness, task relevance, P300, EEG, visual masking

Kokkuvõte

Teadvuse neuuraalsete korrelaatide otsingul: Kas P300 komponent peegeldab teadvuselamust või või teadvustamise tagajärgi?

Laialdaselt aktsepteeritud viis teadvuse teaduslikuks mõistmiseks on uurida teadvuse neuuraalseid korrelaate (TNK), mis on seotud mingi kindla teadvuselamusega. Seda on võimalik teha vastandades omavahel tingimusi, kus mingit kindlat visuaalset stiimulit teadvustatakse või ei teadvustata. Senised uuringud on aga andnud vastuolulisi tulemusi, mis viitab asjaolule, et antud meetod võib lisaks TNKdele välja tuua ka teadvustamisele eelnevaid või järgnevaid protsesse. Käesoleva uurimistöö eesmärgiks oli uurida kas teadvuse signatuuriks peetav P300 komponent on seotud teadvuselamusega või mõne sellele järgneva protsessiga. Kakskümmend üks tervet katseisikut osalesid EEG katses, kus suurem osa katsekordadest teadvustati stiimulit selgelt ning ainult veerandil juhtudest jäi sihtobjekt teadvustamata. Visuaalse maskeerimise katseparadigmas ei kasutatud eristusülesannet, rakendati ühte ja sama stiimulit ning lisaülesannet. Katses kogutud andmed näitasid, et P300

komponent peegeldab teadvuselamust. Seega toetavad antud katsetulemused teooriat, et P300 on teadvustamise korrelaat.

Märksõnad: teadvuse neuraalsed korrelaadid, teadvuselamus, ülesande olulisus, P300, EEG, visuaalne maskeerimine

Introduction

A central goal in the scientific study of consciousness is to find the minimal set of neurophysiological processes that are jointly sufficient for a particular conscious percept (Crick & Koch, 1990; Aru, Bachmann, Singer, Melloni, 2012). To detect the minimal set of neural processes as the neural correlates of consciousness (NCC) the widely accepted contrastive method is to compare brain activity in conditions where subjects perceive or do not perceive the stimuli. However, despite the apparently straightforward logic of this approach the results are inconclusive and contradictory (Aru et al., 2012). It has been argued that the main reason for why one has failed to find universally accepted signatures of NCC is that the experimental methods typically used to study NCC are not specific for NCC but rather also unravel neural processes that precede or follow conscious experience. In other words, contrasting trials with and without conscious perception of a particular target gives us more processes than just the NCC or the signature of the NCC (Aru et al., 2012; de Graaf, Hsieh, Sack, 2012). Depending on how visual awareness is manipulated and assessed, neural prerequisites (NCC-pr) and neural consequences (NCC-co) may be confused with the NCC proper (Aru et al., 2012; de Graaf et al., 2012; Pitts, Padwal, Fennelly, Martínez, Hillyard, 2014a; Pitts, Metzler, Hillyard, 2014b). For example differences in attention, stimulus expectation, adaptation or storing in working memory could bring out differences between trials with and without conscious perception of a target (Aru et al., 2012).

This argument implies that the contrastive analysis has not offered any conclusive evidence for a consistent understanding of signatures of conscious perception. Hence, one has to re-evaluate the whole literature, as it is not known which part of the previous results reflects NCC and which part corresponds to the prerequisites for or consequences of conscious perception. Further, the fact that we cannot be sure about those previous findings implies that some of the neurobiological theories about consciousness might also be based more on the results reflecting prerequisites or consequences than on those directly associated with the conscious percept.

One of the most popular theories of the NCC is the global neuronal workspace theory (Dehaene, Changeux, Naccache, Sackur, Sergent, 2006; Dehaene & Changeux, 2011; Dehaene, Charles, King, Marti, 2014). According to this theory the key signature of information accessing consciousness is the P300 component of the event related potential (ERP). P300 is a positive component often observed on the parietal electrodes, which starts around 300 milliseconds after stimulus onset. In the present work we argue that the P300

component often claimed to be a key signature of conscious access (Del Cul, Baillet, Dehaene, 2007; Dehaene & Changeux, 2011; Dehaene et al., 2014) might actually rather reflect the consequences of conscious perception. We first try to explain which consequences of conscious perception might be captured by P300. Based on these theoretical ideas we construct an experiment to directly test whether the P300 indexes conscious perception or rather the consequences of it.

Which consequences of consciously perceiving a stimulus might the P300 reflect? For intuitive understandings of these processes consider the experimental situation the subject is in. The perceptual tasks are usually demanding and, as the subjects often report during and after the experiment, frustrating for them because the stimuli are very hard to perceive. It is of course the goal of these experimental setups to keep the subjects well below good recognition performance simply to be able to collect enough trials where the target is consciously not perceived. Thus, in these experiments the subjects perceive the target only very faintly or do not perceive the target stimulus at all for most of the trials. This claim is illustrated by low subjective visibility ratings in many of such experiments (e.g. Table 1 in Aru & Bachmann, 2009a or Figure 2E in Del Cul et al., 2007 for some data). This implies that a trial where the subjects perceive the stimulus more or less clearly is a deviant trial, as it is different from all the trials where the subject has hard time to perceive anything. Thus, these kinds of trials with more or less clear perception might be exactly the kind of stimulation that leads to the classic P300 response: as there are a few trials where subjects clearly perceive the stimulus, these trials elicit the P300, because they are somewhat surprising to the subject. Hence, when in the end trials with and without conscious perception of stimulus are compared, the P300 amplitude will be one of their differences. But not because it is a signature of conscious perception but rather because clearly perceived trials are deviants associated with the classic P300 response.

Hence, the first consequence of conscious perception that P300 might reflect is the surprise response stemming from the fact that clearly perceived trials are rare in common experimental setups used to probe for the NCC. Clearly these claims are speculative at first, but they lead to clear predictions that can be tested in experiments: in a paradigm where most of the stimuli are clearly perceived and only a quartile of trials is associated with not perceiving the stimulus, a stronger P300 should be measured for those trials where the target was not consciously perceived. In this case, the P300 would be more pronounced for trials without conscious perception of the target.

However, this experiment would fail simply because trials with conscious perception could also be associated with other post-perceptual processes beyond the detection of deviants. Indeed, we ourselves think that the main reason why P300 accompanies trials with conscious perception is not because trials with conscious perception are deviants but because in those trials working memory content has to be updated and this process is indexed by the P300 (Polich, 2007; 2012). Namely in most studies (e.g. Aru & Bachmann, 2009a; Del Cul et al., 2007) subjects are in addition to performing the subjective visibility task required to give responses about the identity of the stimulus (so that the accuracy or d-prime etc could be measured). This brings an unwanted confound. When subjects perceive the stimulus consciously, they can identify it and hold its identity in working memory until the response screen appears. However, when the subject does not perceive the stimulus consciously, this memory trace is much more feeble – unconscious memory traces decay fast and are qualitatively different from consciously maintaining information (Greenwald, Draine, Abrams, 1996; Kouider & Dehaene, 2007). Thus, trials with and without conscious perception differ with regard to updating and maintaining information about the stimulus until the response screen appears. Updating working memory content is also known to be one of the fundamental processes underlying P300 (Polich, 2007; 2012) and therefore the P300 will be more pronounced when the stimulus is consciously perceived.

How could this intuition be experimentally tested? At first it seems that if this conjecture is correct, having only a subjective visibility task without a discrimination task would eliminate this difference between trials with and without conscious perception. This is not enough, however, as working-memory update could still occur differently between conditions when many different stimuli are presented. In particular, on the trials where the stimuli are clearly perceived, the identity of the target can be updated in the working memory (Polich, 2007; 2012), whereas this is not the case for the trials without conscious perception. Thus, one should use an experimental task where one and the same stimulus is presented so that no working memory update is necessary.

Therefore, another consequence that P300 might reflect is the update of working memory. To get around this confound, we use an experimental setup where there is no discrimination task and where one and the same stimulus is presented all the time. The subjects are required to respond whether they perceived this stimulus on each trial. To make sure that they are not responding randomly we also include catch trials where no target stimulus is included.

Finally, Michael Pitts and colleagues (2014a; 2014b) have argued that P300 could be related to reporting the contents of consciousness. These authors have elegantly shown that when subjects do not have to provide information about stimuli, i.e. these stimuli are task-irrelevant, P300 is not observed for trials with conscious perception. When the same stimuli are task-relevant, a prominent P300 is measured for trials with conscious perception. In other words, these authors observe that the amplitude of the P300 shows an interaction between conscious perception and task relevance. Pitts et al. (2014a; 2014b) claim that P300 is a consequence of consciousness that reflects the need to report about task-relevant stimuli. In their experiments in the “task-irrelevant” condition subjects simply did not have to report about the stimuli, i.e. those stimuli could have been ignored. In the present experiment we wanted to test for a similar effect by having subjects count either the perceived or the unperceived trials, hence making either perceived or unperceived trials task relevant.

All in all, in this study, to exclude working memory update, we use an experimental setup with no discrimination task and always the same stimulus. We also make sure that there would be more trials where subjects perceive the target clearly and only 1/4 of trials with no conscious perception of the target. Thus, in this study we hypothesize that the P300 component, which is thought to be the key signature of conscious access, might actually rather reflect the consequences of conscious perception. In particular we claim that in our paradigm a stronger P300 should be measured for those trials where the target was not consciously perceived. Finally our hypothesis is that our manipulation of task-relevance through the counting task would interact with the P300 results, thus confirming earlier results (Pitts et al., 2014a; 2014b).

Materials and Methods

Subjects

21 subjects participated in the EEG experiment, of whom 7 subjects participated in the pilot experiment but not in the main experiment. The data from 3 additional subjects were not included in the final analyses due to a low percentage of trials where the stimulus was perceived or a high percentage of trials where the subjects reported to have seen the target for catch trials. The remaining 11 participants (4 male) were 26 - 48 years old (mean = 30.6, median = 28, SD = 7.3). They were considered to be in good health and had normal or corrected-to-normal vision. One of them was left-handed. All the included subjects had 52 - 289 trials in each condition ($m = 142$, median = 145, SD = 34). On the experiment day, participants were instructed to avoid drinking coffee. All participants were recruited as volunteers and gave informed consent prior to the beginning of the experiment. The study was approved by the ethics committee of University of Tartu and the experiment was undertaken in compliance with national legislation and the Declaration of Helsinki.

Stimuli

The experiment was programmed using the Python programming language and the VisionEgg package. The target stimulus was a gray @ sign. The mask consisted of a square filled with random noise. More specifically, on every trial, each of the 255x255 pixels in the mask square was filled randomly with one of 256 possible gray levels. Thus, the mask stimulus was updated for every trial independently. Fig. 1. Depicts the target stimulus and the mask. Stimuli were presented on a light gray background with a luminance of 51.6 cd/m². The luminance of the stimuli was 48.5 cd/m² on average (median = 49.5 cd/m², SD = 1.25 cd/m², range = 46.5 – 51 cd/m²). The size of the target stimulus was 1.8 degrees of visual angle and the size of the mask was 2.7 degrees of visual angle. Prior to the stimulus a fixation cross was presented. The size of the fixation cross was 0.3 degrees of visual angle and its luminance was 11.4 cd/m². And the response screen contained the question “Did you see the target object?” in the Estonian language (luminance of 24 cd/m²).

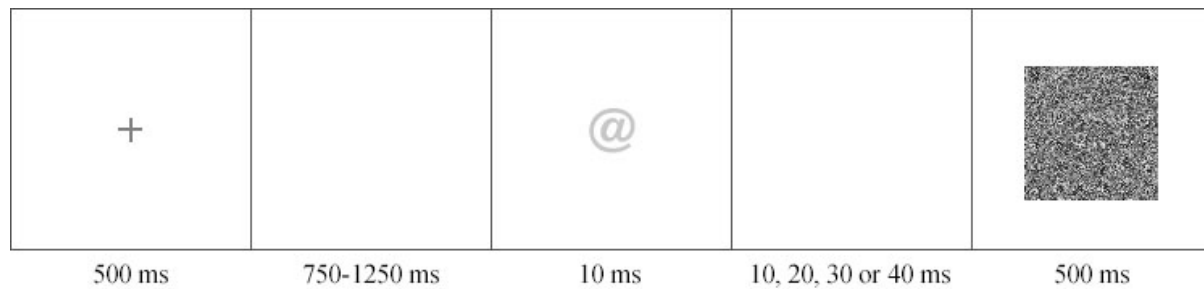


Figure 1. Sequence of the paradigm (fixation cross, blank screen, target stimulus, mask). Images are illustrative and not on the same scale.

In order to achieve the desired rate of perception for the target stimulus two parameters of the experiment were adjusted separately for each participant. First, a suitable inter stimulus interval (ISI) between stimulus and mask was determined. Second, the luminance of the target stimulus was continuously adjusted in order to keep the detection rate approximately at the desired level. The suitable ISI was determined with the help of a short pre-experiment prior to the main experiment. The pre-experiment was very similar to the main experiment (see Task and design) where subjects had to report whether they perceived a stimulus on each trial. Importantly, stimuli were presented with four different ISIs (1-4 frames or 10, 20, 30 or 40 ms) relative to mask onset while the stimulus duration (10 ms) and luminance (48.5 cd/m^2) were kept constant. Every ISI was presented 10 times and 10 additional catch trials where no stimulus was present were also included. After this short pre-experiment resulting detection rates were displayed on the computer screen. The ISI that lead to a detection rate closest to 75% was then chosen by the experimenter for the main experiment for this particular subject. The experimenter also checked whether the amount of false positives for catch trials was sufficiently low.

Because the detection rate for the chosen ISI was rarely optimal (sufficiently closest to 75%) after the first pre-experiment, a second pre-experiment was conducted where the luminance of the target stimulus was varied in order to improve the detection rate. The luminance level of the target stimulus was controlled with the QUEST algorithm (Watson & Pelli, 1983) as implemented by Denis Pelli and made available on the VisionEgg website (<http://visionegg.org/Quest/>). The second pre-experiment was again very similar to the main experiment (see Task and design) comprising 100 trials of which 20 were catch trials. It was expected that at the end of this block the QUEST algorithm would have helped to arrive at an adequate estimate of stimulus luminance that would lead to approximately 75% detection. Indeed, this was mostly the case. Unfortunately, however, in pilot studies it was observed that

detection rates did not stay stable over the course of the main experiment once stimulus luminance was fixed after the second pre-experiment. Thus, we decided not to fix stimulus luminance, but to keep the QUEST algorithm active during the main experiment as well. We hoped that this would lead to a more equal and stable detection rate for all the blocks of the main experiment. In light of this procedure the main purpose of the second pre-experiment was to allow the QUEST algorithm to settle in.

Task and design

Subjects were seated in a dark room, 90 cm from the monitor (SUN CM751U; 1024x768 pixels; 100 Hz refresh rate). Each session began with 2 short pre-experiments to determine the appropriate ISI and target stimulus luminance for each subject (see Stimuli), followed by the main experiment. The main experiment comprised 800 trials in total, 100 in each experimental block. 64 of those trials (8 in each block) were catch trials where no target stimulus was presented. The order of the trials was fully randomized. Each trial began with the presentation of a fixation cross in the middle of the screen for 500 ms (see Figure 1). The fixation cross was followed by a blank screen for a random duration between 750 - 1250 ms. Then the target stimulus was presented in the middle of the screen for 10 ms, followed by a blank screen for a fixed ISI set individually for each subject as described above ($m = 25.5$, median = 30, SD = 9.3, range = 10 - 40 ms). Subsequently, the mask stimulus was presented for 500 ms and thereafter the blank screen again for 500 ms. Finally, the response screen appeared.

Subjects were instructed to fixate on the cross in the middle of the screen, not to blink until the response screen had appeared, and then to report via button press on a standard keyboard whether they had perceived the target stimulus on a given trial (“seen” response) or not (“unseen” response). Seen and unseen responses were given with different hands and there was a break after every 100 trials.

In order to replicate results of recent studies (Pitts et al., 2014a; 2014b), an additional task was given together with the stimulus: to count the number of either seen or unseen trials within a single session. In each experimental session (100 trials), subjects were asked to count alternately either seen or unseen trials. Almost half (6) of the participants began to count from seen and others (5) from unseen trials. Counted number was entered at the end of each session using the keyboard.

EEG recording and preprocessing

The experiments were carried out in a laboratory room equipped with the Nexstim eXimia EEG-system with 60-carbon electrodes cap (Nexstim Ltd., Finland). In every recording, 11 electrodes (PO4, POZ, PO3, P4, P2, PZ, P1, P3, CP2, CP1, CZ) of the extended 10-20 system were used according to the electrode positions (Sharbrough, Chatrian, Lesser, Lüders, Nuwer, Picton, 1991). Simultaneously with the EEG, eye movements were recorded with two extra horizontal HEOG electrodes placed approximately 10 mm from the outer canthis of both eyes. The reference electrode was placed on the forehead, slightly to the right. Through constant monitoring, the impedance at all electrodes was kept below 15 K Ω . The EEG signals were sampled at 1450 Hz and amplified with a gain of 2000. The bandwidth of the signal was ca. 0.1 – 350 Hz.

EEG data were preprocessed with Matlab toolbox Fieldtrip (<http://fieldtrip.fcdonders.nl>; version 01-04-2014). Data were epoched around stimulus onset (-200 to +700 ms). Epochs were baseline corrected with a 100 ms time period before stimulus onset and data were filtered with a 30 Hz low-pass zero phase shift Butterworth filter. All epochs were inspected manually for artifacts and epochs containing blinks, eye movements, strong muscle activity or other artifacts were removed from the analysis. Noisy signals were interpolated with the nearest neighbor method if possible. On average less than 1% of the data were interpolated (median = 0%, SD = 1.1%, range = 0 – 3 %).

Data analysis

The behavioral analysis was carried out with the R programming language (<http://www.r-project.org>; version 0.98.1028). EEG data were analyzed with Matlab toolbox Fieldtrip (version 01-04-2014) as well as with R. Repeated measures ANOVA and paired t-tests were used for both behavioral and mean amplitude analysis. Effect sizes are reported as *ges* (*generalized eta squared*) or Cohen's d, respectively.

Analysis of ERP amplitudes

After the preprocessing, ERP's were computed for all subjects in all conditions (count seen/ seen, count seen/ unseen, count unseen/ seen, count unseen/ unseen). The P300 component was determined on a grand average over all subjects and all conditions. First, the most representative channels for the P300 component were selected. PZ and its surrounding channels (PO4, POZ, PO3, P4, P2, P1, P3) showed the highest P300 amplitudes. Thus, data from these channels were averaged for further analysis. Next, the peak P300 latency was

derived from this grand average. The maximal positive peak was found at 439 ms. Finally, mean peak amplitude was calculated ± 50 ms around the peak latency. In other words, data from each individual ERP were averaged between 389 - 489 ms (439 ± 50 ms) after stimulus presentation.

Results

Behavioral results

The goal of this experimental setup was to keep subjects' detection rate close to 75% and thereby make trials where the target is consciously not perceived deviant trials. In order to achieve the desired detection rate two parameters of the experiment were adjusted. First, the experimenter determined a suitable ISI between stimulus and mask. Second, the QUEST algorithm continuously adjusted the luminance of the target stimulus.

The false alarm rate was satisfactory: 4 % on average (median = 3%, SD = 4%, range = 0 – 14 %). On average the detection rate was 73 % (median = 73%, SD = 5%, range = 67 – 80 %) and it did not vary much depending on the counting condition. The average detection rate for blocks in which seen trials were counted was 74 % (median = 75%, SD = 5%, range = 63 – 80 %). For blocks where unseen trials were counted average detection rate was 72 % (median = 73%, SD = 7%, range = 58 – 80 %). A paired t-test between the mean detection rate for these different types of blocks did not reveal a significant difference ($t(10) = 1.3$, $p = 0.24$, $d = 0.38$).

The detection rates described above are somewhat distorted by some blocks where the detection rate dropped below 60% (9% of all available data). Thus, it makes sense to remove these blocks because they do not fit with the rationale of the experiment. After doing so the average detection rate for blocks where seen trials were counted was 75 % (median = 75%, SD = 4%, range = 69 – 80 %). For blocks where unseen trials were counted the average detection rate was 74 % (median = 75%, SD = 5%, range = 64 – 80 %). Again, a paired t-test between the mean detection rate for these different types of blocks did not reveal a significant difference ($t(10) = 1.3$, $p = 0.21$, $d = 0.4$).

One more aspect to consider was the varying contrast (i.e. the gray level; values ranging between 1-256) of the target stimulus over the course of the experiment. Potentially the contrast could have been different between different conditions. The average gray level over subjects was 204 (median = 207, SD = 12, range = 182 – 219). Within subjects the gray levels varied over conditions 0.38 points on average (median = 0.39, SD = 0.18, range = 0.11 - 0.79). A two-way repeated measures ANOVA was conducted to test whether gray levels are systematically different for seen and unseen trials depending on the counting condition. The main effect for counting condition was not significant ($F(1,10) < 1.0$). The main effect for target detection was significant ($F(1,10) = 9.4$, $p = 0.01$, $\eta^2 = 0.48$). And the interaction between the two factors was not significant ($F(1,10) < 1.0$). The main effect for target

detection may seem problematic, because it could mean that target detection was caused by external factors (i.e. contrast) and the corresponding EEG analyses are not justified. Note, however, that the effect size for this main effect is very small. And indeed the absolute differences in contrast between “seen” and “unseen” trials within subjects are virtually absent ($m = 0.09$, median = 0.05, $SD = 0.11$, range = 0.001 – 0.39). Nevertheless, the significant difference of the contrasts needs to be taken into account in the EEG analysis.

EEG results

The aim of the present study was to investigate whether P300 amplitude is dependent only on conscious perception of the stimulus or whether P300 amplitude also (or even more) reflects post-perceptual processes such as working memory update.

Firstly, two-way repeated-measures ANOVA was conducted on mean P300 amplitudes (see methods for more details). The first factor was counting condition (subject counted seen or unseen trials) and the second factor was target detection (target seen or unseen on a given trial). Fig. 2 depicts the grand average ERP's for all four conditions.

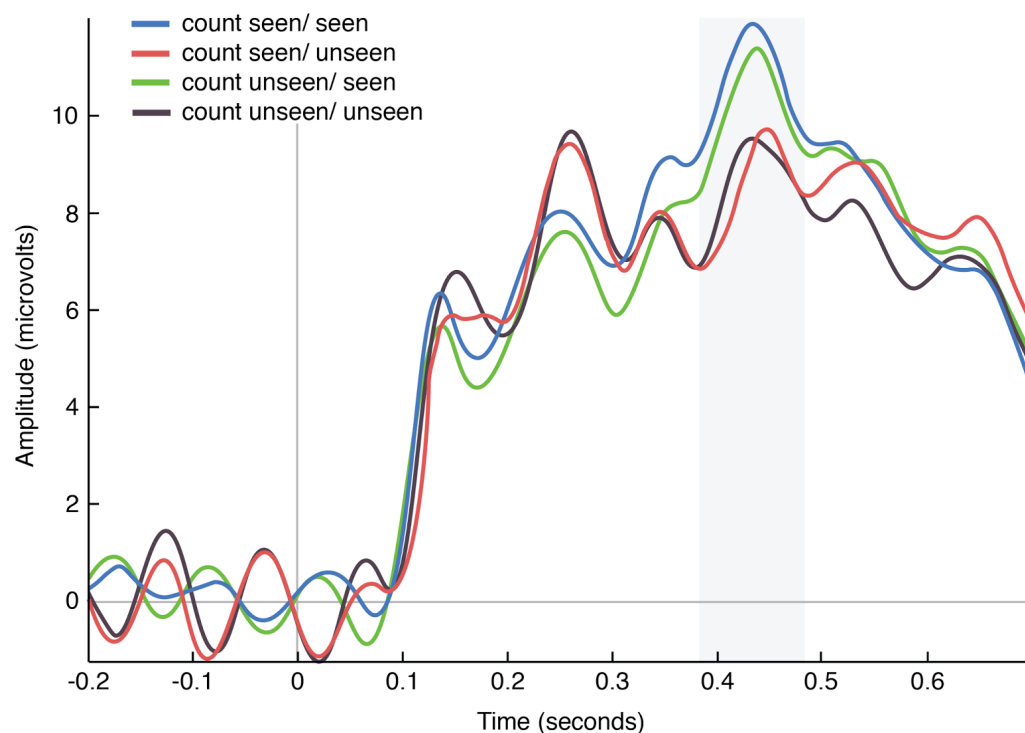


Figure 2. The grand average ERP's for all four conditions (count seen/ seen, count seen/ unseen, count unseen/ seen, count unseen/ unseen) separately. As evidenced on the figure, trials where subjects reported to have seen the stimulus are associated with a higher P300 independently of the counting condition.

The main effect of counting condition was not significant ($F(1,10) < 1.0$) and the main effect for target detection was significant ($F(1,10) = 11.5$, $p = 0.007$, $\eta^2 = 0.08$). However, despite all our manipulations trials with conscious perception (“seen” trials) still were associated with stronger P300 than the trials without conscious perception (“unseen” trials) (Figure 2). Also contrary to our expectation there was no reliable systematic interaction between counting conditions and target detection on mean P300 amplitude ($F(1,10) = 1.5$, $p = 0.25$, $\eta^2 = 0.004$).

The behavioral results showed that there are some differences regarding stimulus contrast between the experimental conditions. In particular, two-way repeated measures ANOVA on the contrast levels showed the main effect for target detection that could have an effect on the EEG analyses described above. To diminish the effects of this potential confound a trial matching procedure was implemented. To equalize counting conditions per subject, matched sets of seen and unseen trials were constructed in a way that each set would have exactly the same contrast level for both seen and unseen conditions. Each of these sets was selected randomly according to the minimum number of unseen responses over all blocks. For instance, if a participant had 10 unseen trials in any block and at the same time it was subject's lowest score over all blocks, and then 10 trials from each block from each condition were used for the matched sets for that participant. In this procedure, only trials with the same contrast level between seen and unseen trials were included in the respective conditions. As a result all four (count seen/ seen, count seen/ unseen, count unseen/ seen, count unseen/ unseen) conditions always comprised an equal number of trial sets (seen and unseen trials with the same contrast) for each subject on each iteration of the set matching procedure ($m = 47$, median = 48, $SD = 11$, range = 24 to 60). As a result, in this analysis we could be confident that there were no variations regarding stimulus contrast between the experimental conditions.

Then, the same analyses were repeated with the matched sets. For the P300 component the results remained the same. The main effect of counting condition was not significant ($F(1,10) < 1.0$) and the main effect of target detection was significant ($F(1,10) = 13.5$, $p = 0.004$, $\eta^2 = 0.09$). Similarly to what was found previously, there was no reliable systematic interaction between counting conditions and target detection on mean P300 amplitude ($F(1,10) < 1.0$). Fig. 4 depicts the grand average ERP's for all four conditions after the trial matching procedure.

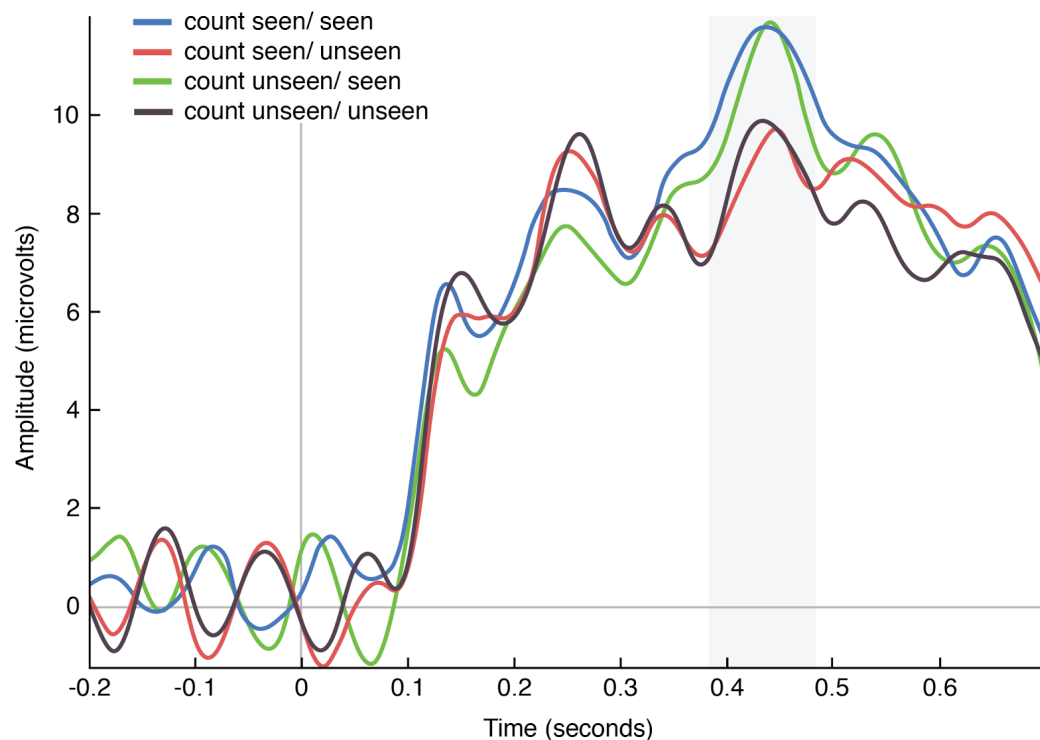


Figure 4. The grand average ERP's for all four conditions (count seen/ seen, count seen/ unseen, count unseen/ seen, count unseen/ unseen) after trial matching procedure. Again, the P300 is stronger for “seen” trials independently of the counting condition.

The data collected did not reveal any impact of the counting task on P300 amplitude. Next we wanted to find out if previous trials had any effect on target detection or counting condition in the following trials. For instance, when subject counted unseen trials and had several unperceived trials in a row, the P300 component could have been feebler since there were no differences between these unperceived trials. Or having a perceived trial after an unperceived trial, while counting unseen trials, could have led to a stronger P300 signal due to a stronger working memory effect (as explained in the introduction).

For this analysis, the first factor was counting condition (subject counted seen or unseen trials), the second factor was target detection (target seen/unseen on a given trial) and the third factor was target detection in previous trial (target seen/ unseen on the previous trial). The results remained largely unchanged: the main effect of counting condition was not significant ($F(1,10) < 1.0$), the main effect of target detection ($F(1,10) = 8.9$, $p = 0.01$, $\eta^2 = 0.06$) was significant and the main effect of target detection in previous trial ($F(1,10) = 1.4$, $p = 0.27$, $\eta^2 = 0.008$) was not significant. And all interactions were also not significant: all F values were smaller than 1.8 and all p values were higher than 0.21. Fig. 5 depicts the grand

average ERP's for all four conditions when subjects counted seen or unseen trials. The results showed that previous trials had no effect on subsequent trials.

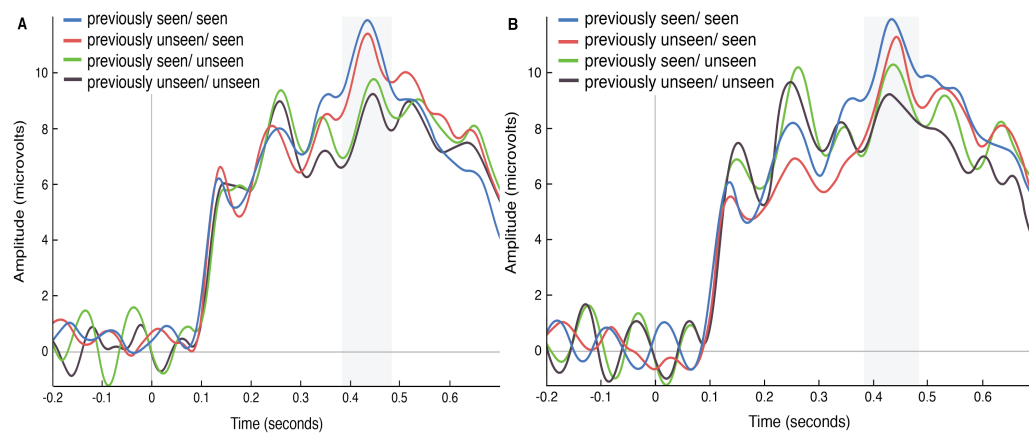


Figure 5. The grand average ERP's for all four conditions (*previously seen/ seen*, *previously seen/ unseen*, *previously unseen/ seen*, *previously unseen/ unseen*) when subjects counted seen (A) or unseen trials (B).

Discussion

The aim of the study was to explore whether the P300 amplitude is a marker of conscious perception of a particular target or whether it rather reflects the consequences of conscious perception, such as working memory update. It was hypothesized that in the experiment where only a quartile of trials is associated with not perceiving the target and where the paradigm contains no discrimination task, a stronger P300 should be measured for not consciously perceived trials. Contrary to our expectation, P300 was still stronger for trials where the subjects consciously perceived the stimulus. Furthermore, we also could not replicate the previous results of Pitts and colleagues (2014a; 2014b), as there was no reliable systematic interaction between counting conditions and target detection on mean P300 amplitude. Altogether, contrary to our expectation, the data collected support previous studies in which the P300 component is claimed to be a key signature of conscious access.

Methodological considerations

Why were the results different than expected? First, it is important to contemplate whether our specific implementation of the experimental paradigm could have included any confounding factor that in turn affected the outcome.

In the study, every so often it was difficult for subjects to perceive the stimuli, as they frequently expressed tiredness during the experiment. Although there was a slight break after every 100 trials and a longer interval after 4 blocks to rest the eyes, the test was still challenging and difficult to carry out, as experiments on consciousness commonly are. Note, however, that all participants were recruited among the acquaintances of the experimenter and therefore had a strong coherence with the study. Also, their frame of mind was constantly assessed after every 100 trials and none of the subjects seemed frustrated during or after the experiment, rather opposite. Thus, we have reason to believe that their effort was sufficient. However, having such relatively difficult conditions for conscious perception might also imply that some of our manipulations were not effective. In particular, one of our goals was to make trials without conscious perception (the unseen trials) deviants. Although subjects had objectively relatively high amounts of seen trials, they reported difficulties perceiving the stimuli; hence obviously unseen trials were subjectively not so deviant after all. In hindsight one could conclude that it would have been better to have objectively different perceptual conditions for seen and unseen trials (e.g. by having different ISIs). Objectively different experimental conditions are of course associated with the problem that any differences

between trials with and without conscious perception could be caused by such objective differences in stimulation (e.g. Bachmann, 2009). This problem can be alleviated by proper control conditions whose ERPs are subtracted from the ERPs of the experimental conditions (e.g. Pitts et al., 2014b). Future experiments should directly test this idea by making unseen trials perceptually and subjectively more deviant in the present experimental setup. It is not impossible that then the P300 would indeed be smaller for seen trials than for trials without conscious perception.

Another aspect, which is worth pondering, is the role of attention. Research shows that people make more mistakes or perform their tasks more slowly when they have a dual-task (Aru & Bachmann, 2009b), as attentional resources must be divided among all of the component tasks to perform them. In retrospect, some participants reported that they paid more attention to the counting task, since they felt it needed more attentiveness – missing one trial can lead to the false final score. Thus, their attention was mostly focused on additional counting task competing for limited resources. Concentrating on the counting task could have decreased the effects of our experimental manipulations. A serious shortcoming of the present experimental setup is that we had no condition where the subjects had no counting task. Hence, we cannot directly verify whether the counting task had an effect on our P300 results.

Comparison to the results of Pitts and colleagues

Michael Pitts and colleagues (2014a; 2014b) have argued that P300 could be related to reporting the contents of consciousness. Their first study on this topic adapted the inattentional blindness paradigm (Pitts et al., 2014a) and found P300 reflecting the report about task-relevant stimuli. The results were achieved manipulating with visual awareness and task-relevance independently while having no additional task (e.g. counting task) beside target detection, as we had here in the present study. Briefly, the paradigm included three experimental phases. First, subjects followed a task-relevant stimulus while a task-irrelevant stimulus was also shown. After the first phase subjects were asked about the task-irrelevant stimulus and had to repeat the same task again. At that point, before the second phase of the experiment, all subjects were aware of the task-irrelevant stimulus but did not have to provide any information about that. Lastly, subjects were asked to follow the stimulus that had been task-irrelevant until then, so that it then became task-relevant. The results showed that P300 was absent following consciously perceived but task-irrelevant stimuli and appeared only when these stimuli became directly relevant to complete the task. In contrast to the current

study, subjects in their experiment did not have to follow the additional task (counting task) to make either the perceived or the unperceived trials task-relevant. They simply reported about seeing the task-relevant stimuli while also perceiving the task-irrelevant stimuli.

The experiment of Pitts et al. (2014a) provides evidence that P300 is a consequence of consciousness that reflects the report about task-relevant stimuli. However, it should be noted that subjects in their study might have ignored the task-irrelevant stimuli every now and then since the trial-by-trial assessment of awareness is not possible in the inattention paradigm. Therefore, one could argue that the experiment of Pitts et al (2014a) did not provide convincing data about the task-irrelevant conditions, as it is not known how often the subjects perceived the task-irrelevant stimulus.

Another experiment designed by Pitts and colleagues (2014b) used a manipulation of awareness and task-relevance in a backward masking task to compare brain activity on aware and unaware conditions depending on task-relevance. In short, two different masking delays (stimulus durations) were used – one leading to 0% awareness and another to 100% awareness of stimulus. All three stimuli (task-relevant, task-irrelevant, control), identical with their previous study, were presented in turns within one block. Subjects had to press a response button whenever they perceived the task-relevant stimulus. The task-relevant stimulus varied by blocks; therefore, on separate blocks of trials the same stimulus was either task-relevant or task-irrelevant. All this allowed comparison of 4 types of trials, as well as our experiment did: aware/ task-relevant, aware/ task-irrelevant, unaware/ task-relevant and unaware/ task-irrelevant. As the P300 was very weak during the task-irrelevant conditions (Pitts et al., 2014b), these results agree with their previous study and support the claim that the P300 component is likely to reflect post-perceptual processes.

This approach seems promising, but yet again, it follows the same concern: it is not known how often the subjects indeed perceived the task-irrelevant stimulus since they simply did not have to report that. One can agree that it is very likely that subjects indeed perceived the task-irrelevant stimuli. However, importantly, perception of the task-relevant and task-irrelevant stimuli could have been qualitatively different: for the task-relevant stimuli subjects could have had a clear conscious percept whereas for task-irrelevant stimuli the experience could have been much more vague. Thus, the results about task-irrelevant conditions are rather conjectural and it is possible that the differences in the P300 still reflect differences in conscious perception.

In contrast to the current study subjects in their experiment did not have a dual-task (e.g. counting task) to make either the perceived or the unperceived trials task-relevant.

Bearing this in mind, subjects in Pitts' et al. (2014a; 2014b) studies might have had more attentional resources for the perceptual task.

The second major difference to point out concerns the influence of decision making processes. In our experiment, subjects were required to respond whether they consciously perceived the target stimulus on each trial. Performing this task required a decision about perceiving or not perceiving the target stimulus, which in turn demanded significant attentional resource. Under some conditions, attention affects detection sensitivity (Smith & Ratcliff, 2009) and having a dual-task might have decreased the attentional effect on target detection. Also, in our experiment all stimuli were complicated to perceive whereas in the masking experiment of Pitts et al. (2014b) stimuli were either clearly visible or completely invisible. Hence, in their experiment subjects essentially had no decision to make about whether they perceived the stimulus or not. In our experiment, however, on most trials subjects had to ponder whether they consciously perceived the target or not. It is to be noted that our experimental paradigm is in this sense much more similar to the experimental paradigms usually applied to study the NCC. The main advantage of the current experimental setup is that subjects had to give answers about conscious perception on every single trial. Hence, we suggest future studies to test the present experimental setup while having objectively different perceptual conditions for seen and unseen trials. Making consciously perceived and not perceived trials objectively different would also eliminate the difficulties in decision making about seeing or not seeing the stimuli. This could be achieved by using two different ISIs - one leading to 0% awareness and another to 100% awareness (as Pitts et al., 2014b did). And to deal with the concern of dual-task, one could use a different manipulation of task-relevance that would be less demanding on the attentional resources of the subjects. It is possible that with these adjustments, our experimental manipulations would reveal a stronger P300 for unseen trials.

P300 as the component of consciousness

One of the important goals in the scientific study of consciousness is to distinguish those brain signals (NCC) that are together necessary for a particular conscious percept from processes that precede or follow the conscious experience. "Experimental findings imply that most of the brain's computations can be performed in a non-conscious mode, but that conscious perception is characterized by an amplification, global propagation and integration of brain signals" (Dehaene et al, 2014). In the light of this global neural workspace theory it is intuitive that conscious access would be associated with late electrophysiological events

such as the P300. Experimental evidence from the group of Stanislas Dehaene has supported this intuition. For example using the backward masking paradigm, Del Cul and colleagues (2007) found P300 component to be most likely associated with subjective perception, because its amplitude as a function of SOA (stimulus onset asynchrony) exhibited the same sigmoidal shape as the proportion of seen trials. Furthermore, when trials with and without conscious perception were compared, P300 was one of their significant difference at a fixed SOA (Del Cul et al., 2007).

Subsequent works, however, have suggested that the P300 amplitude might rather reflect the consequences of conscious perception, such as working memory update, differences in attention, stimulus expectation, adaptation or storing in working memory (Aru et al., 2012). Some studies have even provided evidence to support this idea (Pitts et al., 2014a; 2014b).

Does the P300 component reflect consciousness of the target? The current study was designed to include the following methodological considerations to demonstrate that P300 component is more likely related to the consequences of conscious perception than conscious perception itself: we used no discrimination task, always the same stimulus was presented and the additional task was given together with the target stimulus. Also, there were only 1/4 of trials with no conscious perception of the target.

Despite our efforts to disprove the claim that P300 is a key signature of conscious access, our results actually support the view that P300 is a marker of conscious perception. In this study, the P300 component was found to distinguish aware from unaware trials, and its amplitude was stronger when subjects consciously perceived the stimuli. As we set out to show that P300 is a consequence of conscious perception and failed, the results reported here contradict the proposal that the P300 reflects consequences of consciousness and are in line with the interpretation for P300 as it has been suggested in the global neural workspace theory. Further research needs to show whether the factors discussed above might have derailed us from our mission.

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